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Stabilization of refrigerated avocado pulp: Effect of *Allium* and *Brassica* extracts on enzymatic browning

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ABSTRACT

The aim of this paper was to study the effectiveness of *Allium* and *Brassica* extracts to inhibit the evolution of enzymatic browning of avocado pulp tissue stored at refrigeration temperature (4 °C). Color parameters and mathematical modeling were employed to determine browning kinetics. In general, the addition of *Allium* extracts to avocado pulp was more effective in delaying luminosity decrease during the first days of storage, compared to *Brassica*. Scallion, onion, white cabbage and cauliflower inhibited polyphenol oxidase (PPO), and avocado paste, could be preserved for more than 30 days. Brussels sprouts treatment generated the highest browning kinetic constant (0.86) and conserved acceptable color for less than 10 days. Scallion and garlic showed the great antibrowning indexes (0.96 and 0.77 respectively) considering L^* decrease. Multivariate analysis indicated that vegetable extracts tested could be grouped by variety according to polyphenol content, and by their influence on PPO activity. The results allow the conclusion that polyphenol content of vegetables extracts influence the change of color variables during the first days of storage, while PPO activity influence these parameters in the last period of refrigeration.

1. Introduction

Processing of fresh cut vegetables and fruits promotes their physiological deterioration, biochemical changes and microbial degradation, even when minimal processes are performed.

Enzymatic browning can be a serious problem in fruit, mushrooms, potatoes and other vegetables, producing color alterations that reduce the commercial value of these products, or even make them unacceptable to the consumer. Avocado (*Persea Americana* Mill.) is a climacteric fruit highly susceptible to enzymatic browning and has been the focus of several articles that evaluated synthetic antioxidant compounds, different storage temperatures or atmospheres (Oms-Oliu et al., 2010; Soliva, Elez, &Martin, 2001) in order to avoid browning.

It is well known that the intensity of browning is influenced by the amount of active enzymes and polyphenols in the fruit/vegetable tissue (Gómez-López, 2002; Rice-Evans, Miller, & Paganga, 1997). In most fruits, the levels of phenolic substances are dependent on numerous factors, such as variety, maturity state, or environmental factors (Robards, Prenzler, Tucker, Swatsitang& Glover, 1999). However, fresh-cut fruits are still under study because of the difficulty in preserving their fresh-like quality during prolonged periods. On the other hand, there is an increased concern caused by traditional food preservatives, reporting of occasional allergic reactions in sensitive individuals that generated a restriction or ban in the use of sulphites (Gendel, 2012).

In the last decades, an increased interest in natural compounds displaying antimicrobial effect, inhibiting spoilage or avoiding oxidative processes for preventing the quality loss of minimally processed products was observed (Kyung, 2012; Rojas-Graü, Sobrino.López, Tapia, & Martin-Belloso, 2006; Roldán, Sánchez-Moreno, Ancos& Cano, 2008; Zocca, Lomolino&Lante, 2010). *Allium* and *Brassica* vegetables by-products have been the focus of some research that investigated their potential as natural anti-browning agents in different matrices (Kim, Kim & Park., 2008; Lee, 2007; Rojas-Graü, Soliva-Fortuny& Martin-Belloso, 2008; Roldán et al., 2008; Zocca et al., 2010). Although the antioxidant activity of fresh *Allium* and *Brassica* vegetables was already documented (Leelarungrayub, Rattanapanone, Chanarat&Gebucki, 2006; Patras et al., 2011; Posedek, 2007), there is no enough data concerning the antibrowning potential of different species. In addition, the use of vegetable extracts with recognized preservative properties for the inhibition of avocado browning has not been previously reported. Stabilized by-products from processing *Allium* and *Brassica*

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vegetables could represent a source for obtaining natural antioxidant/antibrowning food ingredients.

The objective of present research was to evaluate effectiveness of *Allium* and *Brassica* extracts to inhibit or delay avocado pulp browning and polyphenol oxidase (PPO) activity.

2. Materials and methods

2.1. Antibrowning Allium and Brassica extracts preparation

Fully mature *Allium*: garlic (*Allium sativum*), onion (*Allium cepa*) and scallion (*Allium fistulosum*); and *Brassica*: white cabbage (*Brassica oleracea*var. viridis), cauliflower (*Brassica oleracea*var. botrytis) and Brussels sprouts (*Brassica oleracea*var. gemmifera) vegetables were purchased from the local market the day after harvest. The vegetables were washed, peeled when necessary, and cut into pieces. The pieces were homogenized and extracted under constant agitation in 0.2 mol/L phosphate buffer solution pH 6.0 (ratio 1:2) at 50 °C for 1 h according to Bustos, Agudelo-Laverde, Mazzobre&Buera(2014). Briefly, the extracts were sterilized at 121 °C for 5 min to avoid further enzyme activity, centrifuged at 15,585 g for 30 min at 4 °C (centrifuge 5804 R, Eppendorf, Germany), and filtered on filter paper (20–25 μm, Whatman ECN- 512-1026). Trehalose was added to the liquid extracts at a final concentration of 15 g/100 ml in order to obtain a physically adequate dry matrix (Schebor, Mazzobre, & Buera, 2010). Aliquots (40 ml) of the extracts were distributed in plastic trays (1 cm height) and frozen at −20 °C for 48 h, further cooled under liquid nitrogen and freeze dried (ALPHA 1-4 LD2 Martin Christ Gefriertrocknungsanlagen GMB, Germany) for 48 h.

Additionally, total phenolic content was determined by the Folin-Ciocalteau method, using gallic acid as calibration standard, as previously reported (Bustos et al., 2014), and expressed as mg gallic acid per gram of extract.

2.2. Avocado puree preparation and storage conditions

Avocado var. Hass pieces of uniform size and color were purchased at commercial maturity in a local market and immediately processed. Fruits were peeled and the pulp was pieced and ground with a food processor HR 1372, 700 W (Philips Electronics, Slovenia). The color values for avocado pulp without any treatment at time 0 were: $L^* = 68.4$, $a^* = -13.3$ and $b^* = 39.6$. All samples were treated with citric acid to decrease the pH of the pulp to 4.0 according to Soliva et al., (2001) thus preventing the proliferation of sulphite-reducing clostridia. The lyophilized vegetables extracts were added to a final concentration of 10 g/100 g. The antibrowning agent used as reference (200 μg/g ascorbic acid − AO) was the same proposed by Soliva et al., (2001). Two control samples were prepared, with citric acid (CA) and without its addition (untreated). After homogenization, avocado purees were distributed in polyethylene bags (50 g) and browning progress and PPO activity were evaluated on each aliquot, during storage at 4 °C for 30 days.

2.3. Color measurement

Changes in color of avocado purees were determined by image analysis using a computer vision system (CVS) according to Agudelo-Laverde, Schebor&Buera (2013), inside a standardized gray chamber (N7 in the Munsell color space). The selected illuminant was D65. A high-resolution (10.1 mega-pixel) digital camera, an EOS 40D (Canon Inc., Japan) was used, with an EF-S 60 mm f2.8 macro lens (Canon Inc., Japan). Samples in glass plates were placed in the standardized chamber and images acquired (white background) at 0, 3, 5, 10, 15, 20, 25 and 30 days of storage at 4 °C. Color images were obtained in Lab values using Adobe Photoshop CS4 software (Adobe Systems Inc., San Jose, CA) and then converted to the standard CIELAB space using mathematical formulas described by Papadakis, Abdul-Malek, Kamdem, & Yam, (2000). From the CIELAB coordinates (L^* (luminosity), a^* (green-red coordinate) and b^* (yellow-blue coordinate)), the color functions total color (ΔE), chroma (ΔC_{ab}) and hue angle (h^*) have been calculated according to the following equations:

$$\Delta E = \left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)^{0.5} \quad (1)$$

$$\Delta C_{ab} = \left(\Delta a^{*2} + \Delta b^{*2}\right)^{0.5} \quad (2)$$

$$h^* = \arctan\left(b^*/a^*\right) \quad (3)$$

where ΔE is total color difference, including luminosity; ΔC_{ab} represents the changes of the chromatic aspects and h^* provides information of the color quadrant in which a given analyzed sample is located, which represents the hue. All functions were calculated taking the respective sample at time zero.

In order to determine the effectiveness of selected extracts, an Anti-Browning Index (ABI) was calculated:

$$\mathrm{ABI}(a^*) = \left(\Delta a^*_{\mathrm{CA}} - \Delta a^*_{\mathrm{extract}}\right)/\Delta a^*_{\mathrm{CA}} \quad (4)$$

Where Δa^* are the total greenness change during storage of citric acid (CA) or extracts treated samples. The greenness used to calculate ABI was selected on the basis of the highest degree of change, so that, could represent better the browning of avocado pulp. CA sample was selected in the calculations in order to evaluate the antibrowning effect of extracts besides citric acid addition.

2.4. Kinetic model for luminosity decrease

Luminosity (L^*) values were fitted to one-phase decay function using ORIGIN 8.0 software (Origin-Lab Corporation, Northampton, MA, USA) (Eq. (5)).

$$L^* = L^*_\infty + \left(L^*_0 - L^*_\infty\right)e^{-\mathrm{k}t} \quad (5)$$

where L^* and L^*_0 are the current and initial luminosity values, respectively, L^*_∞ is the plateau value of the L^* upon prolonged storage, t is the storage time and k is the kinetic constant.

2.5. Determination of PPO activity

PPO extraction and activity measurement were performed according to Soliva et al., (2001). Briefly, 25 g of avocado pulp was mixed with 25 ml of McIlvaine buffer solution at pH 6.5 (1 mol/L NaCl and 5 g/100 ml polyvinylpolypyrrolidone were added to buffer). The homogenate was centrifuged at 2000 g for 30 min at 4 °C (centrifuge 5804 R, Eppendorf, Germany). Three phases were obtained after centrifugation: a hydrophobic liquid phase (avocado oil) on the top of the centrifuge tubes, an insoluble suspension in the middle and a hydrophilic liquid phase (enzymatic aqueous extract) at the bottom of the vials, as was previously found by Saha et al., (2012). PPO activity was determined in the aqueous enzymatic extract using catechol as substrate. One unit of PPO activity was defined as a change in absorbance at 410 nm/min and milliliter of enzymatic extract measured in an UV–visible spectrophotometer Jasco V630 (Jasco Corporation, Japan). The initial rate of the reaction was computed from the linear portion of the plotted curve.

Table 1
Samples and variables codes used in multivariate analysis.

Samples/Treatments	Code	Variable	Code
Untreated	–	Total polyphenol content	TPC
Citric acid	CA	Enzyme activity at day 5	PPO-5
Ascorbic acid	AO	Enzyme activity at day 30	PPO-30
Garlic	G	Anti-Browning Index relative to L* at day 5	ABI-L* (5)
Onion	O	Anti-Browning Index relative to L* at day 30	ABI-L* (30)
Scallion	S	Anti-Browning Index relative to a* at day 5	ABI-a* (5)
White cabbage	Wc		
Cauliflower	C		
Brussels sprouts	Bs		

The results were expressed as relative activity (RA%), calculated as: $RA = 100^{*}A/A_0$, where A is the initial PPO activity and A_0 is the enzymatic activity at a given time.

2.6. Statistical analysis

All samples were prepared in duplicate; each replicate was quantified in duplicate. Results were analyzed by the adjustment to a model with fixed effects for a classification factor with nine levels (treatments and controls). The model included a variance function to take account the presence of an increasing variability pattern related with medium levels of response variable. The adjustment was carried out using an implementation in Infostat (Di Rienzo et al., 2012) of gls function from the nlme library (Pinheiro, Bates, DebRoy& Sarkar, 2012) of R (R Core Team, 2012). The variance function applied was a function of implementation of power variance varPower() from nlme library. These type of statistical analysis allows to compare the effect of treatments and storage time at the same time. Results of the analysis were compared by the DGC means-comparison test (Di Rienzo, Guzmán,& Casanoves, 2002) with a degree of significance of $p = 0.05$.

Finally, hierarchical cluster and principal component analyses were run using the same software. For all analyses, the matrix of the quality variables listed in Table 1 was used. Cluster analysis calculates the distances between all samples using a defined metric such as Euclidean distance. In hierarchical clustering clusters are formed sequentially, been the most similar objects first grouped, and these initial groups are merged according to their similarities (Patras et al., 2011). Pearson correlations were calculated for variables used in multivariate analysis.

3. Results and discussion

3.1. Browning evaluation

Refrigerated avocado pulp became visually darker and less green with increasing storage time (Fig. 1). A control sample of avocado pulp, with no additive (untreated), was also analyzed in order to evaluate if citric acid affected avocado browning. With the aim to compare antibrowning effectiveness of extracts with a recognized antioxidant (Soliva et al., 2001), a sample treated with ascorbic acid was also analyzed.

Luminosity (L^*), yellowness (b^*) and redness (a^*, which increase represents the greenness loss) of avocado pulp samples at different refrigerated storage times are shown in Fig. 2(A–F). In the untreated samples, refrigeration storage caused a luminosity (L^*) and yellowness (b^*) reduction during the first 2 weeks and then both variables arrived to an almost constant value (Fig. 2A and B). Greenness was almost completely lost as perceived visually (Fig. 1), associated to chlorophylls degradation occurring during avocado browning (Watada, Abe, & Yamuchi, 1990). Since the negative values of a^* correspond to a green coloration, the visually perceived changes were in accord with the increase of the a^* coordinate towards the red zone ($a^* > 0$). Pulp with citric acid addition (CA) showed a lower L^* decrease than untreated sample (Fig. 2A). In these samples green (Fig 2B) and yellow (Fig 2C) components also

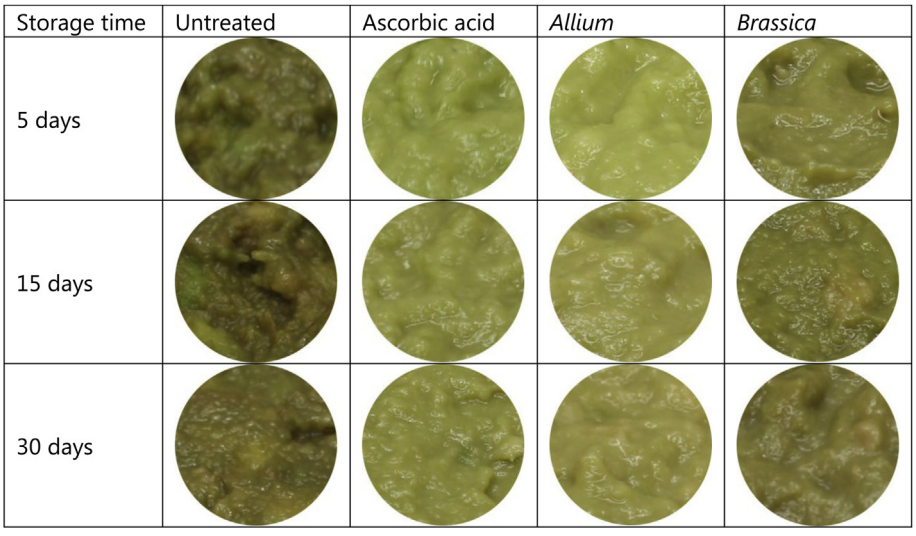

Fig. 1. Images of untreated avocado, and treatments with ascorbic acid (AO), with *Allium* extract (scallion), and with *Brassica* (white cabbage) extract.

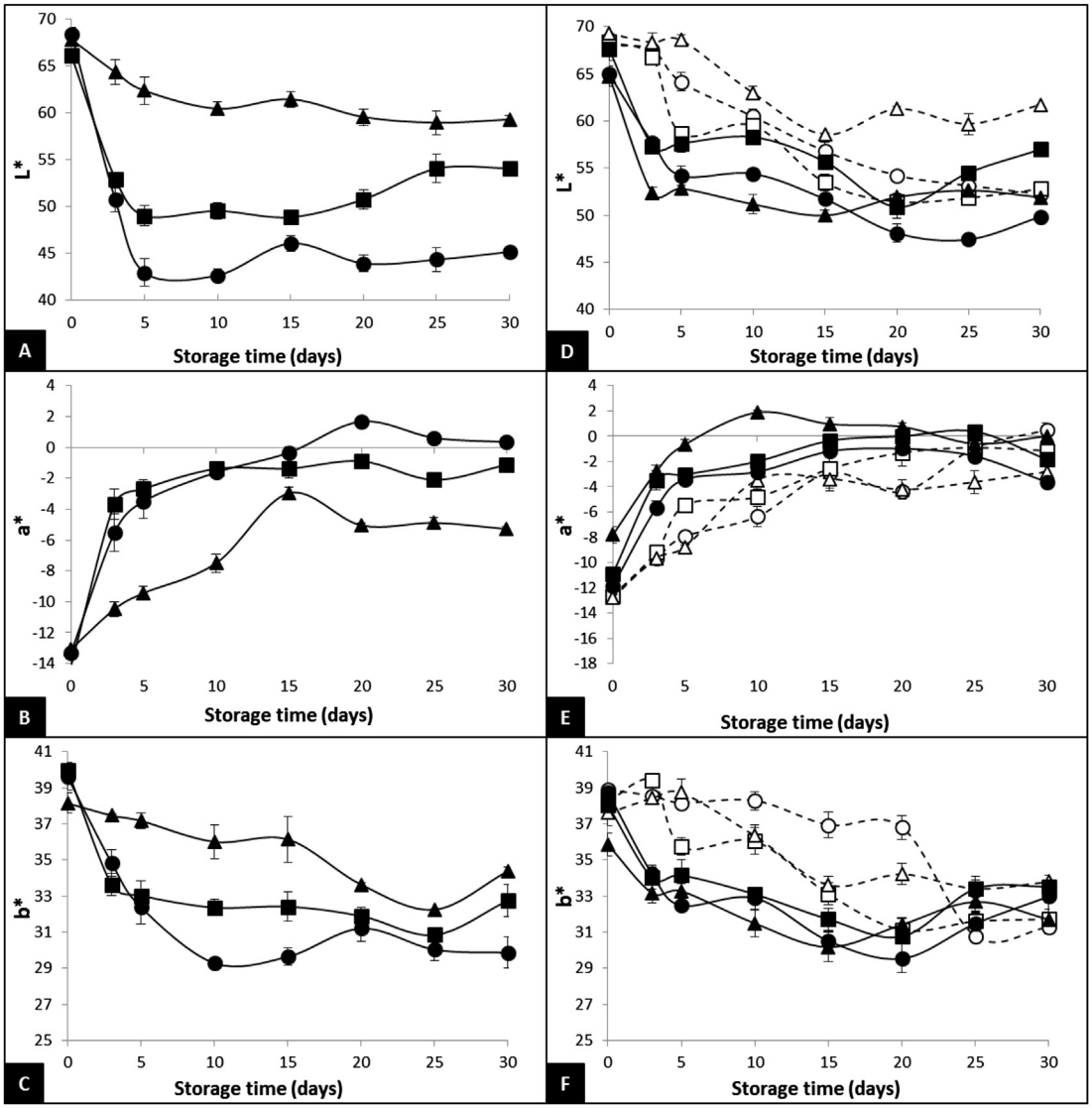

Fig. 2. Lightness- L^* (A–D), greenness decrease-a^* (B–E) and yellowness-b^* (C–F) of refrigerated avocado samples. Key of symbols in figures A–C indicates, ●: untreated, ■: citric acid treated sample, ▲: antioxidant treated sample and in figures D–F: dot lines indicates *Allium* treated samples and continuous lines indicates *Brassica* treated samples: ○: garlic, □: onion, △: scallion, ●: white cabbage, ■: cauliflower and ▲: Brussels sprouts.

showed a decreased rate of change at the last stages of storage. In addition, in CA sample the yellow component decreased up to the 5th day of storage and maintained an almost constant and higher value, in comparison with untreated samples in which b^* continued to decrease until the 10th day (Fig. 2B). However, in both samples (untreated and CA) the same profile of high rate of a^* changes during the first 5 days was observed (Fig. 2C).

In general, the addition of *Allium* extracts to avocado pulp was effective in delaying luminosity decrease during the initial days of storage, compared to the *Brassica* extracts (Fig. 1). However, the L^*values at the final storage time were similar (Fig. 2D) to those of the rest of the samples. On the other hand, *Brassica* extracts promoted an important L^*decrease during first 5 days of refrigeration (Fig. 1).

Scallion extract addition clearly retarded the changes of all color parameters in avocado pulp, promoting a luminosity decrease similar to that found with ascorbic acid addition (AO). These observations indicate that scallion extract behave as antibrowning agent beyond the effect of citric acid addition (Fig 2D). Yellowness and greenness decrease rates (Fig. 2E and F) were slightly higher than in samples containing AO, even that an additional antibrowning effect of scallion extract was detected in comparison with CA pulp ($p < 0.05$).

Although garlic extract was previously recognized as antioxidant and antibrowning (Leelarungrayub et al., 2006; Queiroz, Ishimoto, Bastos, Sampaio, & Torres, 2009), the avocado pulp containing this extract suffered a significant L^*decrease, not as pronounced as in the CA systems, but with a retarded rate of change (Fig. 2A and D). A significant effect in chromatic parameters (b^* and a^*), similar to that observed in the AO samples, was generated by garlic extract addition up to 15 days of storage, reaching similar values to CA at the final storage time (Fig. 2E and F).

Onion extract was slightly less efficient than garlic, especially at the beginning of refrigeration (Fig. 2D), and the systems reached a L^* value lower than that of CA-treated avocado and with delayed yellowness decrease. The green component loss (i.e. the increase of a^* values) was considerably reduced by onion extract until 15 days of storage (Fig. 2B–E).

Table 2
Change in color parameters observed in avocado pulps at 5, 15 and 30 days of refrigerated storage*.

	Storage time (days)	Untreated	CA	AO	*Allium*			*Brassica*		
					G	O	S	Wc	C	Bs
					(10 g/100 g pulp)			(10 g/100 g pulp)		
ΔE	5	28.3^{a}	21.8^{b}	6.7^{f}	6.2^{f}	12.4^{e}	4.4^{g}	15.1^{d}	13.3^{e}	14.2^{d}
	15	27.7^{a}	22.8^{b}	12.4^{e}	15.6^{d}	18.8^{c}	14.9^{d}	18.9^{c}	17.1^{c}	18.2^{c}
	30	28.3^{a}	19.2^{c}	12.2^{e}	21.9^{b}	20.5^{b}	13.1^{e}	18.2^{c}	14.7^{d}	15.6^{d}
ΔC_{ab}	5	12.3^{b}	13.5^{b}	3.8^{d}	4.8^{d}	7.7^{c}	4.4^{d}	10.6^{b}	8.8^{c}	7.6^{c}
	15	16.4^{a}	14.9^{a}	10.7^{b}	10.9^{b}	11.4^{b}	10.3^{b}	13.5^{b}	12.3^{b}	10.5^{b}
	30	16.1^{a}	15.0^{a}	8.7^{c}	15.2^{a}	13.2^{b}	10.7^{b}	10.1^{b}	10.2^{b}	8.9^{c}
h^*	0	108.6^{a}	109.5^{a}	108.9^{a}	108.0^{a}	108.2^{a}	108.8^{a}	107.0^{a}	105.9^{a}	102.3^{b}
	5	96.1^{d}	94.6^{d}	104.2^{b}	101.7^{b}	98.6^{c}	102.7^{b}	95.9^{d}	95.0^{d}	91.1^{e}
	15	90.6^{e}	92.3^{e}	94.8^{d}	94.8^{d}	94.3^{d}	95.7^{d}	92.2^{e}	90.6^{e}	88.2^{f}
	30	91.1^{e}	91.9^{e}	98.7^{c}	89.1^{f}	92.0^{e}	94.7^{d}	96.3^{d}	93.1^{e}	89.9^{f}

* The values with different superscripts in the same line or column differ significantly ($p < 0.05$). ΔE and ΔC_{ab} parameters were calculated considering time 0. Samples and variables codes are listed in Table 1.

Except for the systems containing Brussels sprouts extracts, that presented initial a^* values around −8.0, addition of all *Allium*, white cabbage and cauliflower extracts to avocado pulp did not affect a^* initial values, that ranged between −11.0 and −14.3 (Fig. 2E).

The complementary changes in color functions: color difference (ΔE), chroma (ΔC_{ab}) and hue angle (h)* at 5, 15 and 30 days of refrigeration are shown in Table 2.

A *hue* value of 91.1 was obtained at 30 days of storage of untreated avocado, corresponding to a yellow coloration, in agreement with browning development. Total color change found in untreated and CA samples were mainly associated to the difference found in L^*, while chroma (ΔC_{ab}) and hue angle (h^*) presented similar rate of change ($p > 0.05$), due to the slight differences observed in the green component (Table 2).

It is remarkable that in *Brassica* treated pulps after 10 days of storage parameters a^* (Fig. 2E), b^* (Fig. 2F), and ΔC_{ab} (Table 2) tended to rise, with only small changes in hue (h^*) value. Considering *Allium* extracts, this trend was less obvious, particularly in garlic treated avocado that showed the highest change in ΔC_{ab} and h^* (Table 2).

Lopez-Malo, Palou, Barbosa-Cánovas, Welti-Chanes, & Swanson (1998) have established that an a^* value higher than −0.5 defines the sensory acceptability limit of avocado pulp color. Thus, the storage period necessary to develop an unacceptable color change was defined on this basis and presented in Table 3. Additionally, the antibrowning index and the total polyphenols content associated with vegetable extracts are shown in the same table. The a^* negative values for ascorbic acid-treated pulp (typical of a green coloration) indicated that refrigeration storage maintained the samples at acceptable values for at least 30 days (Table 3). Although the browning progress in CA pulp was faster than in AO treated sample (Fig. 2A–C), considering only the green component loss, both treated pulps could be conserved for more than 30 days at 4 °C (Table 3). These results agree with those by Soliva et al (2001) who found slight differences between both antioxidants.

Scallion extract presented similar ABI-a^* compared to AO sample until 15 days of refrigeration (Table 3), while ΔE and ΔC_{ab} of garlic-treated pulp were similar to AO sample until the same period (Table 2). However, at 30 days of refrigeration the green component was completely lost and *hue* decreased to less than 90, indicating that garlic extract allowed avocado pulp conservation under 4 °C for 25 days (Table 3). Contrary to the observations for the garlic treated sample, onion treated pulp ($h^* < 90$) should be stored for less than 30 days in order to remain within acceptable values (Table 3).

Compared to onion-treated samples, garlic extract treated pulp presented a higher ABI-a^*until 15 days of storage. However, with further storage time both extracts reached a similar browning degree than CA, reflected by ABI value close or lower than zero (Table 3).

White cabbage treatment slightly affected L^*compared to CA with a lower ΔE value until 15 days of refrigeration (Table 2). This result is due to the fact that yellowness was unaffected by cabbage extract addition (no differences with CA) and green component loss was retarded ($p < 0.05$, Fig. 1C and D). Thus, ΔC_{ab} was also reduced, h^* was above 90 and avocado with white cabbage addition could be stored at 4 °C for one month. As observed for garlic and onion treated samples, an increased browning occurred after 15 days of storage of this cabbage-treated avocado pulp compared to CA sample (Table 3).

Cauliflower-treated avocado showed a decreased rate of yellow component loss, in contrast with cabbage treated avocado samples ($p < 0.05$, Fig. 2C and D). Greenness change presented the same trend than cabbage-treated samples (similar ABI-a^*),

Table 3
Antibrowning indexes of *Allium* and *Brassica* extracts at 5, 15 and 30 days of refrigerated storage, and storage time limit of avocado pulp*.

	Storage time (days)	Untreated	CA	AO	*Allium*			*Brassica*		
					G	O	S	Wc	C	Bs
					(10 g/100 g pulp)			(10 g/100 g pulp)		
Limit storage time (days)		15–19	>30	>30	25–29	>30	>30	>30	>30	<10
ABI a^*	5	0.15^{c}	–	0.68^{a}	0.59^{a}	0.37^{b}	0.65^{a}	0.27^{c}	0.32^{c}	0.38^{b}
	15	−0.01^{d}	–	0.21^{c}	0.24^{c}	0.22^{c}	0.27^{c}	0.17^{c}	0.18^{c}	0.32^{c}
	30	0.03^{d}	–	0.40^{b}	−0.003^{d}	0.12^{c}	0.24^{c}	0.37^{b}	0.31^{c}	0.40^{b}
TPC (mg GA/g)		–	–	–	1.54^{c}	1.47^{c}	2.58^{a}	0.74^{d}	0.71^{d}	1.76^{b}

* The values with different superscripts in the same line or column differ significantly ($p < 0.05$). Samples and variables codes are listed in Table 1.

Table 4
Kinetic parameters of the modified first-order fractional model fitted to luminosity decrease of untreated and vegetables extracts treated avocado pulp stored at 4 °C.[a]

Kinetic parameters	Untreated	CA	AO	G	O	S	Wc	C	Bs
L_0^*	68.5	66.1	67.8	69.1	69.1	70.6	64.9	67.5	64.7
k	0.870	0.540	0.183	0.050	0.105	0.104	0.255	0.444	0.866
Plateau	44.2	51.2	59.5	46.6	50.7	59.4	51.4	55.3	51.7
R^2	0.956	0.850	0.924	0.977	0.911	0.812	0.819	0.874	0.955

[a] Samples codes are listed in Table 1. L_0 = initial luminosity value and k: kinetic constant.

which was lower than CA (Fig. 2E and F), indicating that cauliflower extract affected both chromatic variables changes, allowing to preserve the acceptable avocado color for more than 30 days (Table 3).

Brussels sprouts-treated samples presented a similar effect on delaying browning development than cauliflower treated samples, especially considering L^* (Fig. 2B) and b^* (Fig. 2D). The green component loss was retarded in these samples with effectiveness comparable to that of AO (Fig. 2C). That means Brussels sprouts extract showed more effectiveness in delaying browning compared to CA. However, due to the a^* initial value in this sample was higher than pulp with other crucifer extracts, the greenness was completely lost after 15 days of storage, limiting the acceptability of pulp (Table 3).

3.2. Luminosity decrease fitting

As shown in Fig. 2, the general shape of the curves for L^* consisted of an initial phase of fast decrease (up to the 5th day), followed by a phase of slow decrease, approaching an almost constant value (or plateau). The luminosity decrease can be considered as a good index of browning increase. In Table 4, browning kinetic rates for untreated avocado pulp and *Allium* and *Brassica* treated samples, derived using the modified first-order fractional conversion model (Eq. (5)) for luminosity decrease, are shown. In all cases, good correlation coefficients (above 0.8) were found between the experimental and calculated values.

The highest kinetic constant for browning development was found for untreated pulp and Brussels treatment which agrees with the earlier greenness loss observed in both samples (Table 4). In CA avocado pulp, the browning was retarded, as discussed before. Bates (1968) also found that pulp acidified with lemon juice browned less rapidly than the untreated sample. In addition *Allium* extracts generated a great decrease in the darkening rates (k), which were lower than in AO systems. Particularly, for garlic extract the lowest k was observed, in agreement with the great browning delay observed up to 15 days of refrigeration. However, this effect could not be extended beyond 15 days and even negative ABI values were found at 30 days. On the other hand, *Brassica* extracts addition decreased the darkening kinetic constants, compared to CA sample (except for Brussels sprouts), being white cabbage the most effective antibrowning agent with a kinetic constant slightly higher than AO (Table 4).

Among all the evaluated pulp samples, those containing ascorbic acid and scallion extracts showed the best antibrowning properties (highest L^* plateau values) and also the best preserved green and yellow components, while untreated avocado and garlic were the ones with the highest rate and degree of browning (lowest L^* plateau value). In onion, cabbage and Brussels sprouts extracts the calculated plateau values for L^*were quite similar to CA (Table 4).

3.3. PPO activity inhibition

Avocado pulp without any treatment presented a significant increase of relative PPO activity, particularly at the last days of the analyzed period at which enzyme activity increased almost 50% (Fig. 3). In agreement with our results, Soliva et al. (2001) and Pinheiro et al. (2012) observed that avocado pulps, without any chemical treatment and stored under a normal environment presented higher pulp darkening and higher PPO activity.

The addition of citric acid caused no significant differences with untreated avocado until 15 days of avocado pulp refrigeration (Fig. 3). This agrees with previous observations on PPO inhibition occurring only at high concentrations of citric acid related to the phenolase Cu-chelating power (Bates, 1968; Pizzocaro, Torreggiani, & Gilardi, 1993).

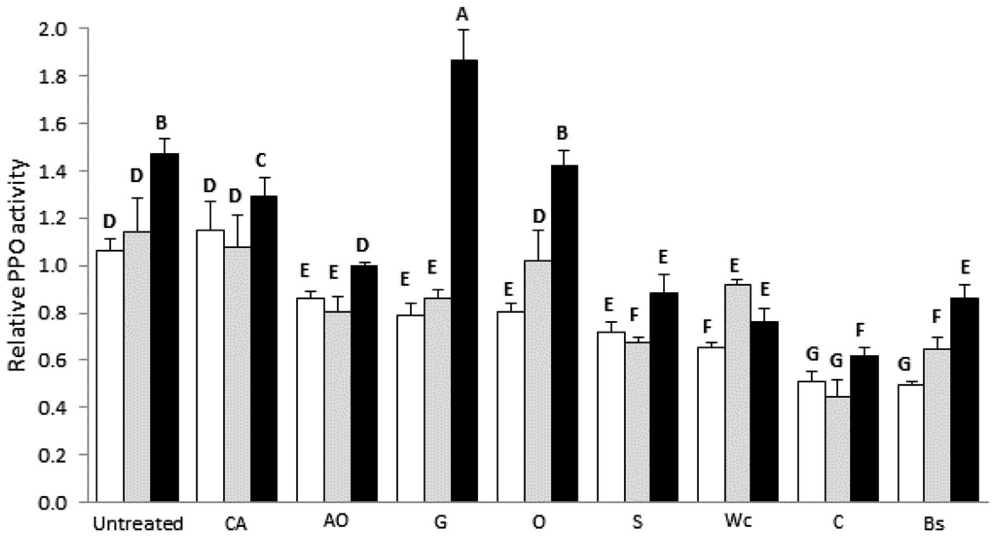

Fig. 3. Relative polyphenol oxidase activity during 5, 15 and 30 days of avocado storage at 4 °C. Samples codes are listed in Table 1.

All *Allium* and *Brassica* extracts showed inhibited PPO activity up to 15 days of refrigeration. Particularly scallion and all crucifer extracts maintained lower enzyme activity up to one month of storage compared to time zero (Fig. 3).

The observed antibrowning effect is caused by the reduction of the orthoquinones generated by the action of the enzyme on phenolic compounds (Gómez-López, 2002; Soliva et al., 2001), so that slightly inhibition of relative enzyme activity was observed until 15 days of refrigeration in the extract-treated systems (Fig. 3).

With garlic and onion addition, enzyme activity in avocado pulp was inhibited until 2 weeks of storage at 4 °C, after this period the activity increased to similar values observed in untreated or CA samples (Fig 3). These observations are in agreement with Roldan et al. (2008), who found that different onion by-products inhibited PPO activity from avocado pulp in an *in vitro* study. Avocado pulp treated with garlic extract presented a PPO activity increase more than 50% (relative to time zero) after 15 days of storage, at which the complete green component loss was detected (Table 3). On the other hand, scallion was the only *Allium* extract that showed PPO activity inhibition at all evaluated storage times, especially at the initial storage stages.

Considering crucifer vegetables, white cabbage was the less effective in preventing enzyme activity. However, an important decrease of enzyme activity was detected at the first storage days (Fig. 3). Cauliflower was highly effective as PPO inhibitor, with 50% of initial activity. In addition, Brussels sprouts extract generated a slightly lower effect on enzyme activity related to green component loss before 2 weeks of storage (Table 3).

The differences found between *Allium* and *Brassica* species could be attributed to the quality and/or quantitative aspects of antibrowning compounds (Cartea, Francisco, Soengas, & Velasco, 2011; Lanzotti, 2006) in both kind of vegetables. In this regard, *Allium* vegetables have been characterized as a good source of low molecular weight organosulfur compounds with thiol groups (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002; Negishi, Negishi, & Ozawa, 2002, Perez-Gregorio, García-Falcón, Simal-Gándara, Rodrigues, & Almeida, 2010) which are proposed as responsible of PPO inhibition (Kim et al., 2008).

Although the precise mechanisms for the enzymatic antibrowning effects exhibited by *Brassica* vegetables are not well-understood this family is known to contain organosulfur glucosinolates (Volden et al., 2008; Volden, Borge, Hansen, Wicklund, &,

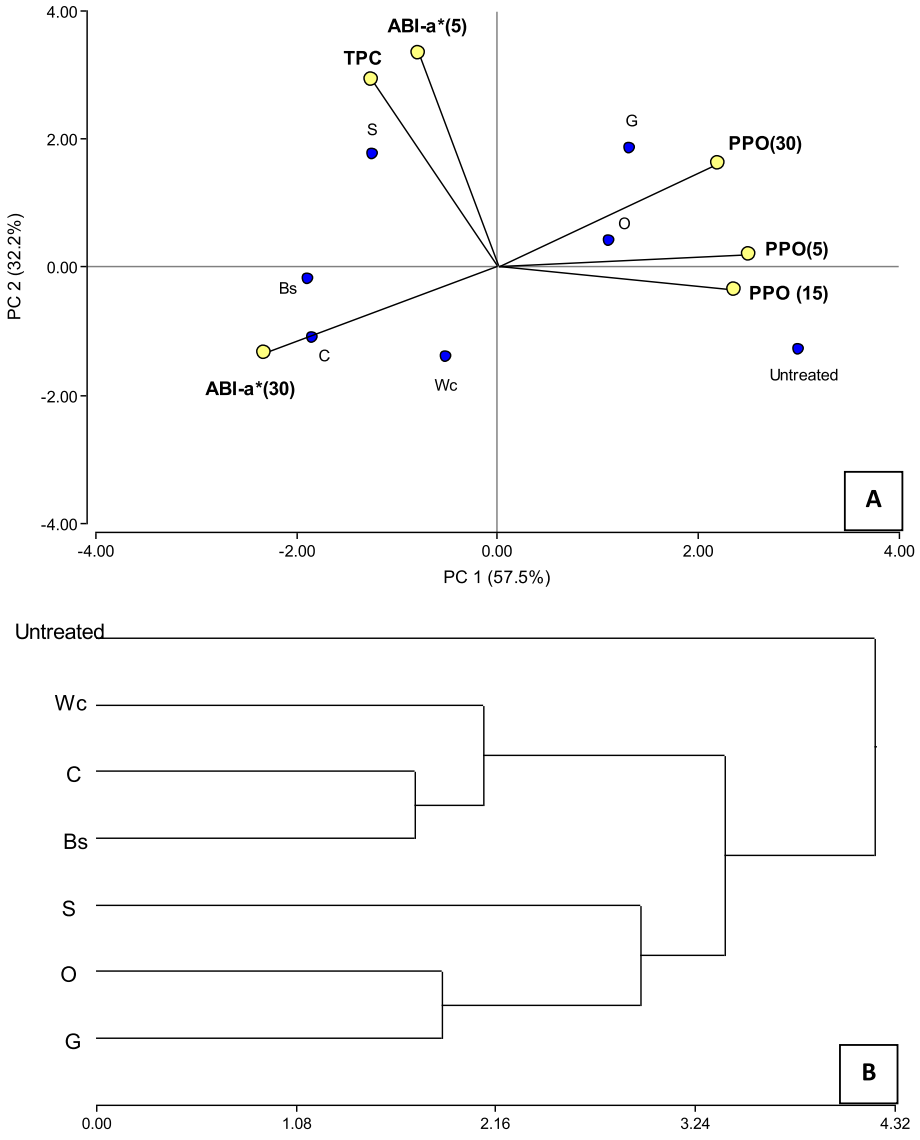

Fig. 4. Score plot of PC1 vs. PC2 from selected data in PCA analysis (A) and dendrogram obtained from cluster analysis (B). Samples and variables are coded as informed in Table 1.

Bengtsson, 2009) which have been shown to effectively inhibit PPO activity. Other natural components such as organic acids, phenols and anthocyanins may also contribute to the PPO inhibition (Zocca et al., 2010).

3.4. Multivariate analysis

Principal components (PCA) and hierarchical cluster analysis (Fig. 4) were performed on the polyphenol content from extracts (TPC, Table 3), antibrowning index (ABI-a^*) (Table 3), and PPO activity at 5, 15 and 30 days of storage at 4 °C (Fig. 3). In the analysis, AO and CA samples were excluded in order to compare only between the effects of extracts addition to avocado pulp at pH 4.0 and untreated pulp.

PCA (Fig. 4A) showed that samples were jointed together according to the vegetable species of the extracts employed, on the two first components which represent the 89.7% of the total variance, with a cophenetic correlation of 0.987. PCA is particularly useful in examining correlations among variables in the original dataset, since it chooses the new axes to lie along directions of highest correlation (Patras et al., 2011). That means, significant Pearson correlations were found.

ABI-a^* in scallion treatment was closely related to polyphenol content, while in garlic and onion extracts-containing systems, which presented a similar behavior, ABI-a^* was more related to PPO activity. In addition, from principal components biplot was clear that extracts polyphenol content did not affect enzyme activity and highly influence greenness lost at first days of storage.

Polyphenols content (TPC) of the selected extracts was strongly related to antibrowning index at 5 days of avocado pulps storage (0.87, $p < 0.0001$). It is remarkable that ABI-a^* at final stages of storage period analyzed (30 days) correlated negatively with polyphenol oxidase activity (PPO) at 5 (-0.82, $p = 0.0003$), 15 (-0.60, $p = 0.0238$) and 30 (-0.90, $p < 0.0001$) days of avocado pulp refrigeration, meaning that greenness loss is related with enzyme activity. Also, significant correlations were found between PPO activities at different refrigeration storage time (data not shown). Thus, enzymatic activity can explain the browning development at the longest storage times. This can be attributed to the presence of natural inhibitors or endogenous antioxidants that are consumed at the first stages. It is also noticeable that the phenolic compounds that are related to the browning inhibition at the first stages may also become substrates of PPO at the later stages. Other factors, such as those related to lipid oxidation, may account for the lack of correlation between PPO and the degree of color changes.

Cluster analysis allowed to group samples with the highest correlations together while samples with small correlations were widely separated. Results from cluster analysis are represented in a dendrogram of Fig. 4B which clearly reflects that avocado pulp treated with selected extracts showed differences in considered variables that allowed samples separation in three groups: control, *Allium* and *Brassica* treated samples, with a cophenetic correlation of 0.863 (Fig. 4B). The advantage of cluster analysis is that the different groups obtained considered all the variance in the dataset, as compared to the 89.7% variance represented by our PCs of PCA.

4. Conclusion

Among all the evaluated pulp samples, those containing ascorbic acid and scallion extracts showed the better antibrowning properties and also the best preserved green and yellow components up to 30 days of refrigeration storage. *Allium* extracts, particularly garlic, generated a great decrease in the rate of darkening, but lower than AO, with great browning delay observed up to 15 days of refrigeration.

The PCA and hierarchical cluster analysis were useful for establishing similarities and dissimilarities among the samples in the browning progress of refrigerated avocado pulp with addition of vegetables extracts with recognized antioxidant properties. Multivariate analysis showed that polyphenols content of extracts highly influence ABI-a^* at first days of refrigerated storage, but this index was more influenced by PPO activity at final days of storage. That means, although *Allium* and *Brassica* extracts contain polyphenols that could act as substrates in the enzymatic PPO browning reaction, the inhibitory effect of the extracts prevailed over their reactivity.

This research has shown that by the addition of *Brassica* and *Allium* aqueous extracts it is possible to produce avocado pulp avoiding browning problems during shelf-life, and the product could be kept for more than thirty days, retaining acceptable color properties of such products.

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